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THE DISPOSABLE POROUS BED VISCOMETER:

A NEW METHOD FOR MEASURING BLOOD VISCOSITY

BY

J.P. CROWLEY, J. METZGER, A. GRAY, E. MERRILL AND C.R. VALERI

NAVAL BLOOD RESEARCH LABORATORY
BOSTON UNIVERSITY SCHOOL OF MEDICINE
615 ALBANY STREET
BOSTON, MA 02118

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A disposable porous bed viscometer (PBV) that has rheologic properties related to that of the microvasculature has recently been developed for the measurement of whole blood viscosity (WBV) under conditions of low shear and low stress. The measured end-point of apparent viscosity with the PBV is the time for a given volume of whole blood to flow a calibrated distance through the porous bed. The various conditions of sample collection, sample storage and temperature required to obtain accurate and

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reproducible results with the PBV are described. The mean WBV of 242 normal persons was 22.7 ± 5.3 seconds corresponding to an apparent viscosity of 5.7 ± 1.3 centipoise, a value in general agreement with previous studies obtained using rotational viscometers. There was, as expected, a significant difference in the WBV of normal men and women related to their different packed cell volumes. Platelets and granulocytes also influenced WBV, but in proportion to their contribution to the total packed cell volume. In normal subjects, fibrinogen level did not significantly influence WBV. The PBV is a rapid and reproducible means to measure WBV in normal populations which should have clinical application in disorders in which ease of performance and replication is an important consideration.

ABSTRACT

A disposable porous bed viscometer (PBV) that has rheologic properties related to that of the microvasculature has recently been developed for the measurement of whole blood viscosity (WBV) under conditions of low shear and low stress. The measured endpoint of apparent viscosity with the PBV is the time for a given volume of whole blood to flow a calibrated distance through the porous bed. The various conditions of sample collection, sample storage and temperature required to obtain accurate and reproducible results with the PBV are described. The mean WBV of 242 normal persons was 22.7 ± 5.3 seconds corresponding to an apparent viscosity of 5.7 ± 1.3 centipoise, a value in general agreement with previous studies obtained using rotational viscometers. There was, as expected, a significant difference in the WBV of normal men and women related to their different packed cell volumes. Platelets and granulocytes also influenced WBV, but in proportion to their contribution to the total packed cell volume. In normal subjects, fibrinogen level did not significantly influence WBV. The PBV is a rapid and reproducible means to measure WBV in normal populations which should have clinical application in disorders in which ease of performance and replication is an important consideration.

INTRODUCTION

Human blood has been recognized as a non-Newtonian liquid for several decades (1-4). Guyton (5) has emphasized the relation between whole blood viscosity (WBV) and packed cell volume (PCV) at all flow rates. In addition he noted that as the velocity of flow decreases the viscosity of blood increases dramatically (5). This non-Newtonian behavior has been the subject of much research and speculation. Research on the flow properties of blood have led to both macrorheological and microrheological concepts (2,3), according to which either red cell to red cell interactions, individual red cell deformability or both are considered to be the cause of the non-Newtonian properties of whole blood. This non-Newtonian behavior makes it difficult to predict the viscosity of blood in various parts of the vascular tree. A direct measurement is needed when an estimate of viscosity is required.

Studies on normal human and animal blood with a coaxial cylinder electromagnetic viscometer operating at low rates of shear stress which are relevant to microcirculatory flow have shown the existence of yield shear stress (YSS), a value below which flow of whole blood totally ceases (6,7). These studies (6) have also shown that the non-Newtonian range extends from 0 up to about 10-50 inverse seconds of shear rate. A limitation of these studies was that they were performed under conditions possibly not relevant to the microcirculation which consists of branching, interlaced capillaries rather than one channel of fixed and constant width.

The purpose of the present report is to describe a disposable porous bed viscometric (PBV) device. The PBV was designed to evaluate the apparent viscosity of blood as it flows through the communicating random channels of a porous bed comparable in pore diameter to the lumens of arterioles and venules (8,9). A previous study in dogs has shown that the flow rates in the PBV are comparable to those observed in microcirculatory vessels (8), e.g. approximately 40 u mean pore diameter at low stress and low shear conditions (8). Since this yields measurements within 3-4 minutes after venipuncture, it allows for use of anticoagulated or non-anticoagulated blood.

METHODS

I. Blood collection and storage

Blood was collected from normal healthy volunteers into plastic syringes. The viscosity was measured immediately on non-anticoagulated blood, and the blood collected into vacutainer tubes containing K3 EDTA or heparin and stored at room temperature for up to 4 hours and at 4 C for 24 hours. In some experiments, the blood stored at 4 C and at 22 C was incubated at 37 C for 30 minutes prior to testing. In other experiments the blood was stored at room temperature prior to testing.

II. Porous Bed Viscometer

A detailed description of the porous bed viscometer (PBV) has been recently published (8). The PBV is a disposable device approximately the size of a ballpoint pen which consists of a porous bed enclosed in a transparent, cylindrical plastic case. The porous bed is composed of sintered polyolefin (Porex®) which has been shown to have communicating random channels that have a mean pore diameter of approximately 40-50 u (8). An upper fitting connects to a transparent capillary tube with a lumen diameter of 1.5 mm. Two marks are inscribed 53 mm apart on this capillary neck, and the time for the fluid meniscus to descend from the first to the second mark is measured.

III. Preparation of Blood Samples with Increased Concentration of Platelets

Plateletpheresis of normal, healthy volunteers was performed using a Haemonetics model V50 Blood Processor. Prior to the

plateletpheresis procedure 30 ml of blood was collected and a 10 ml volume of blood was added to each of three K3 EDTA tubes. The three samples were centrifuged at 180 x g for 5 minutes and the platelet-rich plasma was removed from two tubes, pooled, and re-centrifuged at 1200 x g for 10 minutes to obtain cell-free EDTA plasma. No plasma was removed from the third sample. The plateletpheresis product was centrifuged for 10 minutes at 160 x g to sediment red blood cells and white blood cells, and the platelet-rich plasma (PRP) was removed. A portion of the PRP was centrifuged at 1000 x g for 5 minutes to sediment the platelets. The ACD-A plasma was removed and the concentrated platelets were resuspended in the cell-free EDTA plasma. Platelet-rich EDTA plasma was added to the EDTA red blood cells in two of the three samples to prepare blood samples with increased platelets. The mixtures of red blood cells and plasma were adjusted to prepare three samples with identical total volumes and hematocrit values with increasing platelet concentrations. Platelet counts, hematocrit (Hct) or packed cell volumes (PCV), RBC counts, and WBC counts were measured in the samples.

IV. **Preparation of Blood Samples with Increased Concentration of Granulocytes**

Using a Haemonetics Model 30 Blood Processor, granulocytes were isolated from normal volunteers who were treated with 20 mg of prednisone 15, 12, and 2 hours prior to the leukapheresis procedure. One volume of acid-citrate-dextrose (ACD, NIH, Formula A) anticoagulant supplemented with pentastarch was used to collect 8 to 10 volumes of blood. Thirty ml of blood was obtained from the normal volunteer prior to the leukapheresis procedure and a 10 ml volume of

blood was added to each of three K3 EDTA tubes. These samples were processed as previously described to obtain cell-free EDTA samples.

The leukapheresis product was centrifuged for 10 minutes at 160 x g to sediment the red blood cells and white blood cells and the PRP was removed. The red blood cell-white blood cell pellet was resuspended in saline and 6% dextran with a molecular weight of 500,000 was added to sediment the red blood cells. The white blood cell-sodium chloride mixture was removed and centrifuged for 10 minutes at 180 x g and the supernatant removed. The cells were washed with saline and the granulocytes were resuspended in the cell-free EDTA plasma. White blood cell-rich EDTA plasma was added to the EDTA red blood cells in two of the three samples to prepare blood samples with increased granulocyte concentrations. The mixtures of red blood cells and plasma with increased concentrations of granulocytes were adjusted to prepare three samples with identical total volumes and hematocrit values, with increasing granulocyte concentrations. Platelet counts, PCV, Hct, RBC counts, and WBC counts were measured in the samples.

V. **Preparation of Blood Samples with Increased Concentration of Mononuclear Cells**

Normal volunteers were plateletpheresed using the Haemonetics Model V50 Blood Processor and 30 ml of blood was obtained in K3 EDTA prior to the plateletpheresis procedure. The three 10 ml volumes of K3 EDTA blood were treated to prepare K_3EDTA cell-free plasma as described above. The platelet-rich plasma was centrifuged at 160 x g for 10 minutes, and the cellular residue was diluted with 0.9% sodium chloride to achieve a final volume of 120 ml. A volume of

75 ml of ficoll-hypaque with a specific gravity of 1.077 was added to a ficoll-hypaque plastic bag separation system (Ethox Corp., Buffalo, NY) and the cellular mixture was layered on top of the ficoll hypaque solution and then centrifuged at 258 x G for 30 minutes to isolate the mononuclear cells. The mononuclear cells were removed, diluted with 500 ml of 0.9% sodium chloride and centrifuged at 580 x G for 10 minutes. All the visible supernatant was removed and the mononculear cell pellet was resuspended in K_3EDTA cell-free plasma. The mononuclear cell-enriched EDTA plasma was added to the EDTA red blood cells to prepare blood samples with increased concentrations of mononuclear cells. The red blood cells and plasma with increased mononuclear cell concentrations were adjusted to prepare three samples with identical total volumes and hematocrit values with increasing mononuclear cell concentrations.

VI. **Preparation of Blood Samples with Increased Concentrations of Fibrinogen**

Three hundred-eighty ml of blood was collected from a normal volunteer into K3 EDTA. Three aliquots of 60, 120, and 200 ml were prepared and centrifuged at 180 x g for 10 minutes. The final hematocrit values of the three aliquots were adjusted to 20, 40 and 60% so that all three aliquots contained a final volume of 120 ml. Exogenous fibrinogen was prepared by dissolving the lyophilized human fibrinogen (Calbiochem F341582, Behring Diagnostics, La Jolla, CA 92037) in EDTA cell-free plasma to achieve a fibrinogen concentration of 1400 mg%. The three aliquots were subdivided into three 40 ml samples and exogenous fibrinogen was added to achieve concentrations of 200 mg%, 400 mg% and 600 mg%. Whole blood

viscosity was measured at 22°C and 37°C in samples with hematocrit values of 20, 40, and 60 V% each containing 200 mg%, 400 mg% and 600 mg% fibrinogen.

VII. **Additional Measurements**

Red blood cells and white blood cells were counted on a Coulter ZBI (Coulter Electronics). Total packed cell volume (RBC + WBC + platelets) were measured in some experiments by a standard microhematocrit method. Platelets were counted manually using phase microscopy. Fibrinogen levels were measured on an automated Coag-A-Mate using phase microscopy. Fibrinogen levels were measured on an automated Coag-A-Mate using the thrombin time method. On samples from EDTA blood 15 ul of 0.025 N C_aC1_2 was added to 1 ml of a 1:10 dilution of plasma in veronal buffer to correct for the presence of EDTA. Using this technique, the fibrinogen level was 281.1 ± 91.6 mg/dl, n = 310); this value was not significantly different from the level of 275 ± 75 mg/dl, measured in 0.38% sodium citrate plasma.

VIII. **Whole Blood Viscosity Measurements**

The measurement of whole blood viscosity (WBV) was performed at either 37 C or at room temperature (22 C). The anticoagulated blood samples (EDTA), viscometers, syringes and needles were maintained at a temperature at which the measurements were performed. Blood samples stored at room temperature or at 4 C were rewarmed for 30 minutes in a 37 C water bath prior to WBV measurement. After careful mixing of the sample, the blood was slowly withdrawn from the vented vacutainer into a 10 ml syringe using an 18 gauge needle. The needle was discarded and the blood

sample mixed within the syringe and all entrapped air was removed and then the syringe was attached to the luer-lok fitting of the viscometer. The viscometer was then inverted and the blood was slowly injected into the viscometer. When the blood reached the end of the viscometer, the syringe was removed and the viscometer was placed vertically in a rack in a 37 C water bath or in a rack at room temperature. The blood flowed by gravity through the viscometer into the collection tube. A stopwatch was started when the meniscus of blood flowing through the capillary tube reached the upper calibration mark and was stopped when the blood reached the lower calibration mark. The time in seconds required for blood to flow between the two marks was recorded as the flow time. Duplicate determinations were performed on all samples and the average of the two values was reported as flow time. If the two times differed by more than 2 seconds, a third determination was done and the average of the two closest times reported. The viscometers and syringes were stored at room temperature or warmed to 37 C prior to the testing of the non-anticoagulated blood.

IX. **Statistical Measurements**

Mean and standard deviation (m ± SD) were reported for each set of measurements. The means of experiments were compared by using the paired t-test. Certain measurements were reported as a percentage of another measurement. Linear regression and correlation coefficient were used to measure the relationship between variables. A p value of <0.05 was considered significant for all tests.

The figures indicate the exact number (N) of analyses in each statistical comparison.

RESULTS

Whole blood viscosity (WBV) of blood anticoagulated with EDTA was not significantly different from that of unanticoagulated blood (EDTA = 22.8 ± 5.3 (n=6), Heparin = 23.8 ± 5.6 (n=6), no anticoagulant = 22.2 ± 4.1 seconds (n=6). There was a significant difference when blood was studied at 22°C (~room temperature) as opposed to 37°C, the values being 22.8 ± 5.3 (n=6), and 19.0 ± 2.7 seconds, (n=6) $p<0.05$ respectively. The ratio 22.8/19.0 = 1.20 is less than the ratio of viscosity of water at 22° (0.955 cp) to the viscosity at 37° (0.691 cp), a value of 1.38, indicating that red cell flexibility and red-cell to red-cell interactions did not linearly follow the temperature-water viscosity relation. There was no significant change in the WBV of EDTA anticoagulated blood over a 4 hour period either stored at 37°C or stored at 4°C and then rewarmed to 37°C. The mean WBV of 21 normal bloods stored at 4°C for 24 hours (22.7 ± 4.5) was not significantly different than that of freshly drawn samples (23.0 ± 4.5) when the stored blood was rewarmed to 37°C (Figure 1). The mean WBV of 242 normal persons was 22.7 ± 5.3 seconds (range = 12.5 to 41.5 seconds, Figure 2) corresponding to a value of 5.7 ± 1.3 centipoise (9). The WBV of 41 males was 27.1 ± 5.5 seconds and of 47 females 21.9 ± 4.1 seconds, $p<0.001$.

There was no significant correlation between fibrinogen level and WBV in the 148 normal subjects for whom fibrinogen levels were available (Figure 3). We studied the effect of the addition of exogenous human normal purified fibrinogen on the WBV and did not observe any significant increases in WBV over an approximately fourfold range of

fibrinogen levels (Figure 4) when the PCV was kept constant (40%). Similar results were observed at 22°C.

Platelets added to whole blood to achieve levels approximately 10 and 20 times higher than normal (1.14 ± 6.4 and 2.27 ± 1.55 x 10^6/ul) increased WBV by 110% and 124% respectively (Figure 5). A greater effect was seen when polymorphonuclear granulocytes (PMNG) were added in the same increments (Figure 6). The WBV increased 145% and 234% respectively. An increase of 116.5% and 149% were observed when mononuclear cells were added in increments of 10 and 20 x normal respectively (Figure 7). There was a significant correlation between the WBV and both the granulocyte count ($p<0.01$) and mononuclear cell count ($p<0.01$) between cell counts of 10,000-350,000/ul, (Figure 8). The effect of granulocyte and mononuclear levels on the WBV was proportional to the effect of the addition of these cells to the PCV (Figure 9).

DISCUSSION

This study reports a method of measuring WBV by timing the flow of blood through a porous bed. Several previous methods have been devised to measure WBV using a flow principle but involving capillary tubes of various dimensions (7,10-12). The WBV values obtained in the present study are comparable to values obtained by Letcher et al. (13) using a rotational viscometer, but are somewhat lower than the viscosity values obtained by Reinke et al. (11,12) using vertical capillary tubes even when temperature differences are taken into account. This difference probably relates to the difference in the microrheology of a porous bed from a capillary tube. The data obtained in normal subjects with PBV (22.7 ± 5.3 seconds) when translated to approximate centipoise (5.7 ± 1.3) are comparable to previous values obtained in 11 dogs (9) and are within the reference ranges reported by Kaber et al (14). We did not observe any influence of anticoagulation with heparin or K3 EDTA (Results) which is consistent with previous reports (15-17). There were no significant changes in WBV using EDTA samples kept for 4 hours at room temperature or in storage up to 24 hours at 4 C (Figure 1).

The studies of human blood reported here are in accordance with studies on canine blood by Tu et al. (18) who, using the same PBV report a good correlation between hematocrit and viscosity ($r = 0.81$, $p<0.0001$), but no correlation between fibrinogen and viscosity. The supposed role of fibrinogen as a factor in whole blood viscosity evolved from studies of yield stress in blood. Viscosity is defined as the ratio of shear stress to shear rate. With some non-Newtonian liquids, the

shear stress remains with a non-zero value as shear rate becomes zero: this value of shear stress being the yield stress. Morris et al (19) used an optical non-viscometric technique which took several minutes per reading to study blood samples. They showed that yield stress varied with nearly the second power of fibrinogen concentration, more or less in agreement with earlier studies with a rotational (Couette) viscometer (20-23). Further Merrill showed that viscosity measured at low shear rate (e.g. about 1 sec^{-1} in the same viscometer) varied significantly at fixed hematocrit as fibrinogen concentration varied. As in the studies of Morris et al. (19), significant time was required to determine the shear stress/shear rate relation: about 3 minutes at each fixed rotational speed.

The studies reported herein with the PBV are thus at variance with Morris and with one of the present authors previous studies (20-22) in that no correlation is found between PBV flow time and fibrinogen concentration when samples of the same hematocrit are compared. Recent studies of blood with rotational viscometers are in accordance with the present findings. Pola et al. (24) used a Haake Rotovisko® cone-plate viscometer and reported measurements at two shear rates: 15 sec^{-1} and 90 sec^{-1}, on blood samples from patients in whom fibrinogen concentration had been increased by nearly a factor of two (by response to angioplasty) or decreased by nearly a factor of two (by urokinase treatment) with respect to the pretreatment fibrinogen level. Their data suggested that the influence of fibrinogen on viscosity was marginal as compared with previous observations; indeed increased or decreased fibrinogenemia did not produce a significant viscosity change in these patients. The data of Pola et al.

(24) might be questioned on the grounds that the shear rate was too great to allow observation of the fibrinogen effect (although 15 sec^{-1} is in the range in which Merrill (20-22) observed a strong fibrinogen effect). This issue of shear rate is probably ruled out by the observations of N.K. Man et al. (25), who studied the viscosity of human blood in a Contraves cone-plate viscometer at shear rates of 0.204, 0.512, 0.945, 8.11, 20.41 and 128 sec^{-1}, as well as with the PBV. The fibrinogen concentration in this limited group varied from 191 to 349 mg%. When compared at the same hematocrit in a range of 40 ± 0.9 (M ± SD), the viscosity measured by the Contraves cone-plate viscometer at any shear rate, and the flow time determined by the PBV, bore no correlation with measured fibrinogen level. As to why our recent findings, as well as those of Pola and Man with cone-plate devices are at such complete variance with the earlier studies using the Couette instrument is probably related to differences in the speed with which the viscosity can be measured between the Couette device and the PBV and rotational viscometers. What may happen over several minutes in vitro is discussed in some detail by Reinke et al. (11,12) who measured viscosity by fine capillary tubes of different diameters. They showed that totally different results were obtained when the tube was vertical than when it was horizontal. There was negligible dependence of apparent viscosity on shear rate with vertical tubes, but increasing apparent viscosity with decreasing shear rate in horizontal tubes. Reinke et al. (12) discussed their results in terms of Fahraeus-Lindqvist effects, formation of clear plasma layers, and formation of clusters of red cells mediated by fibrinogen, leading to cores rich in red cells streaming through the plasma layers. Thus in

Reinke's experiments with straight tubes (12), as in early experiments with a Couette viscometer (23), and recent experiments with a tilted plate observed under a microscope (19), it appears that a rouleaux formation evolves in blood with time which is not present in flowing blood or blood that is rapidly tested after loading into the viscometer. Reinke (12) concluded by noting that flow conditions in a complicated network may not be comparable to those in a single unbranched tube. In the vascular system, these workers suggested that frequent vessel branching prevents the development of a fully established marginal plasma layer. They suggested that a "close approximation of in vivo flow geometry" would be required for a definitive in vitro assessment of blood rheology. The PBV may be one sort of approximation. Because of the dividing and recombining channels in the porous bed and variation in channel shape and size, blood is effectively remixed as it flows through the bed and, as in the living microcirculatory vessels, the forcing function is a pressure drop and the responding function is flow rate.

The lack of correlation of fibrinogen level with WBV in normal subjects indicates that in the normal range of fibrinogen and PCV, red cell factors (26-32) rather than the fibrinogen level are more important determinants of WBV. Other factors that could explain the large variation in WBV for a given normal PCV include the various plasma proteins, lipoproteins, lipids, and alterations in RBC membrane fluidity and deformability (33). Thus, the measured WBV as assessed by the PBV is a biological characteristic of an individual sample and cannot be deduced by a simple formula that uses PCV and fibrinogen level to predict the expected WBV. When constituents of

blood other than red cells were added to blood, the WBV increased only in proportion to the increase of the total PCV (Figure 9). Twenty-fold increases in platelets increased WBV by 124% while 20-fold PMNG enrichment provided a 234% increase. These results with normal leukocytes are in agreement with previous studies of Lichtman (34) on blood samples obtained from patients with pathologically increased leukocyte levels. At normal leukocyte and platelet levels, these cells have little impact on WBV.

The results suggest that the PBV may prove useful in a variety of clinical settings, including the evaluation of patients with potentially increased WBV such as in primary and secondary polycythemias (26,30), leukocytosis (35,36) and in shock states with low flow (36). Because it is convenient to use and easy to repeat, the WBV measured with a PBV may also find usefulness in monitoring the effect of therapy on WBV in these various states. For the same reasons it may also be useful in epidemiologic studies (37) to ascertain if WBV can be established as a dependent or independent risk factor for arteriosclerotic vascular disease (19,31,33,38-41), myocardial infarction (13,26,42,43), or stroke (13,44,45). We are currently engaged in one such study on a large normal population in Southeastern New England.

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FIGURE 1: The effects of 24 hour storage of venous blood in EDTA at 4°C with WBV measurement at 37°C.

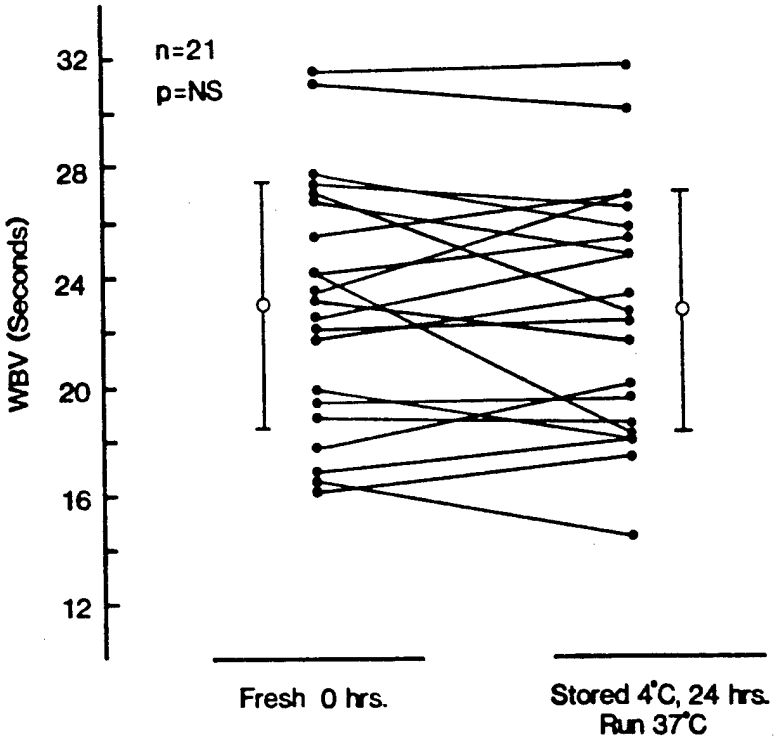

FIGURE 2: Relation of WBV to PCV in 242 normal subjects. The single point at 0% represents the mean of 6 EDTA plasma samples tested on the PBV.

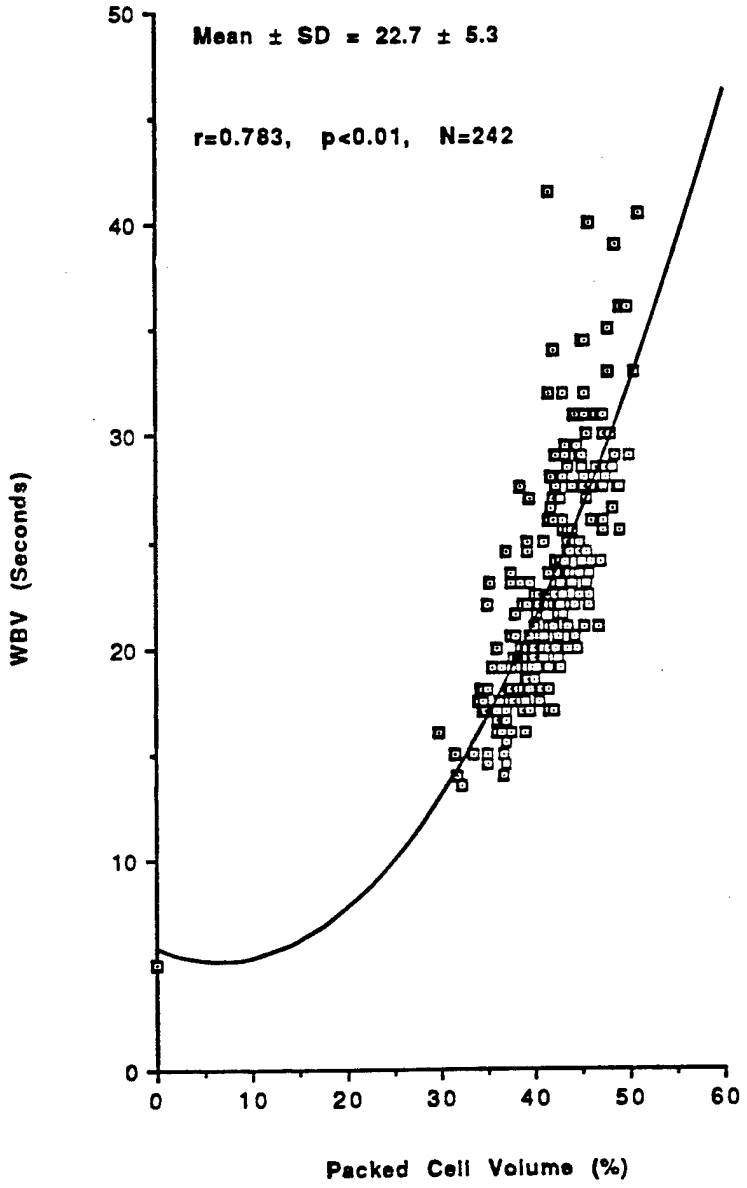

FIGURE 3: Relation between WBV and fibrinogen levels in normal subjects.

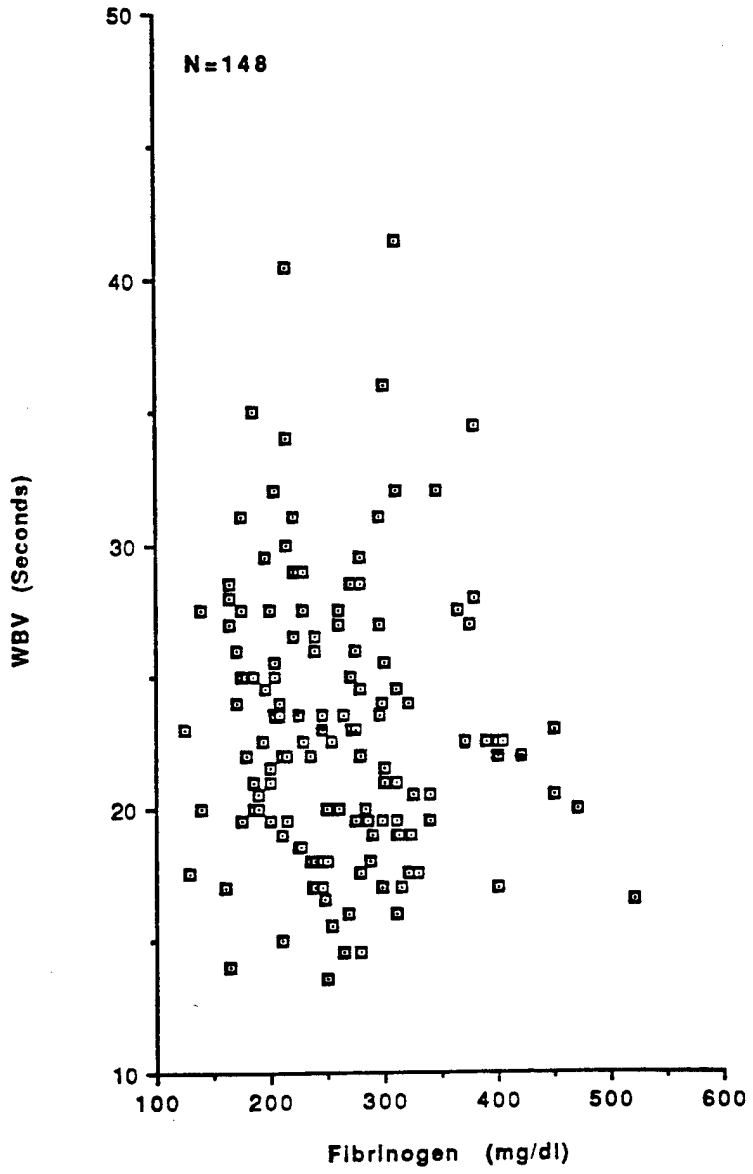

FIGURE 4: The effect of the addition of fibrinogen to the whole blood of normal donors on WBV measured at 37°C.

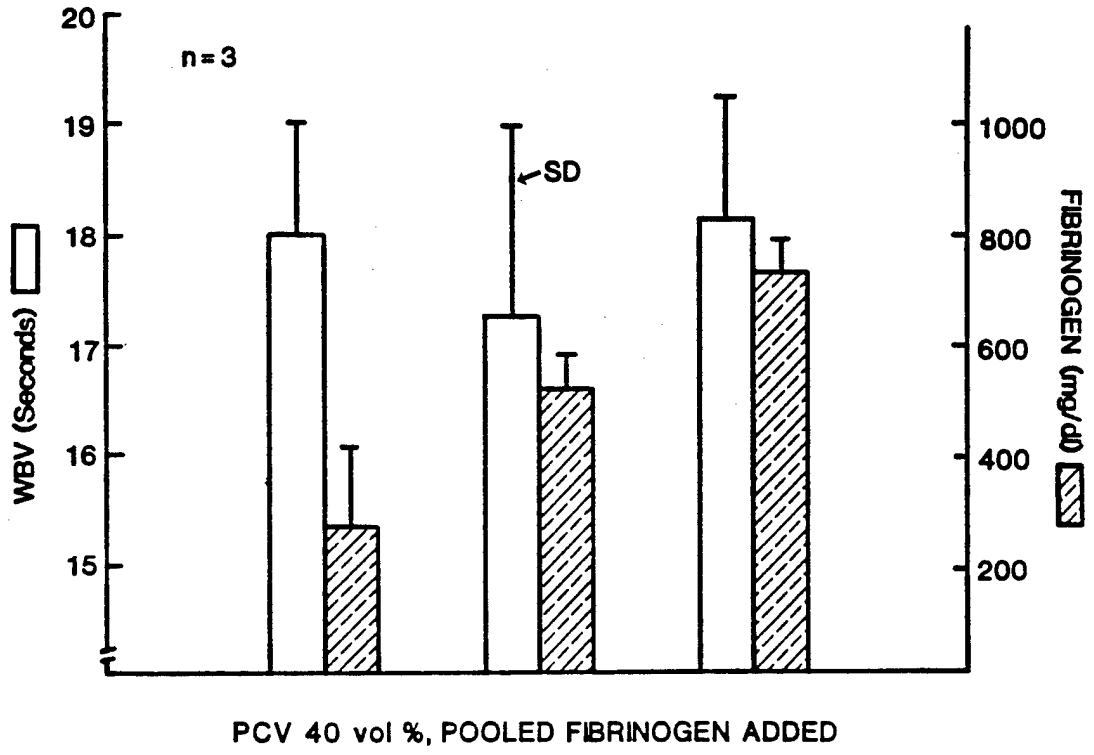

FIGURE 5: The effect of the addition of platelets to the whole blood of normal donors on WBV.

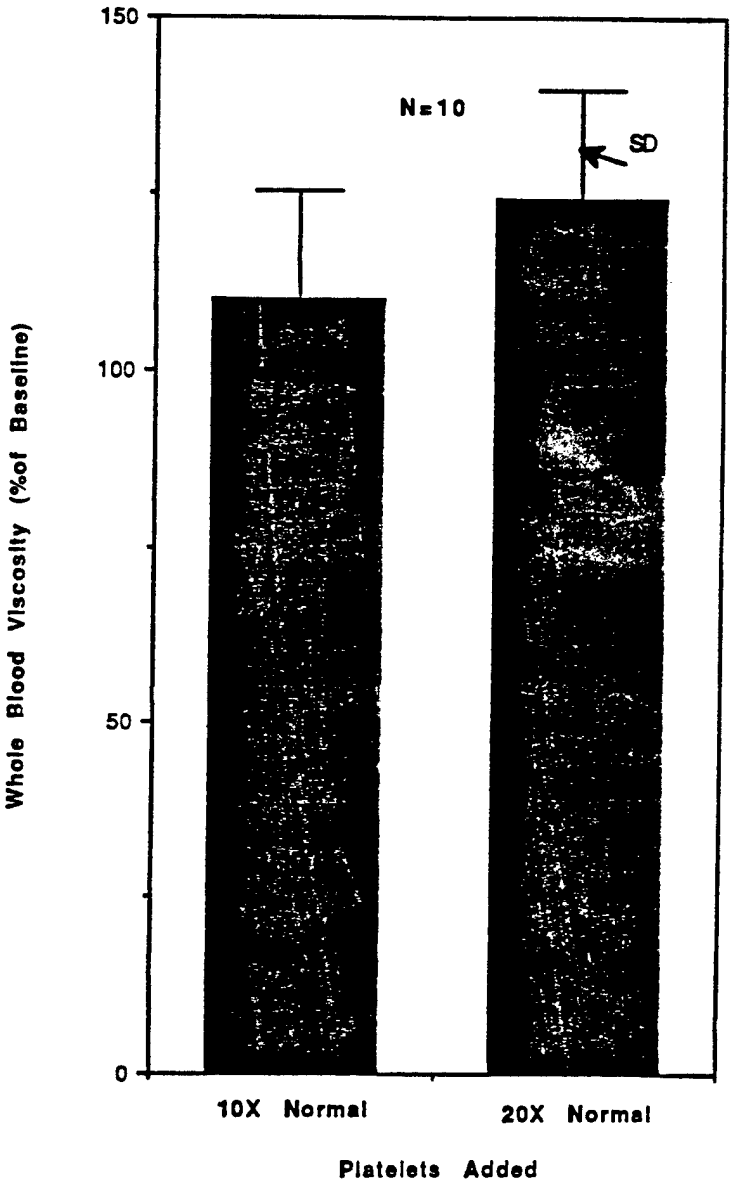

FIGURE 6: The effect of PMN granulocyte levels on WBV. Increased counts were achieved by the addition of purified granulocytes obtained by leukopheresis and then added to normal blood.

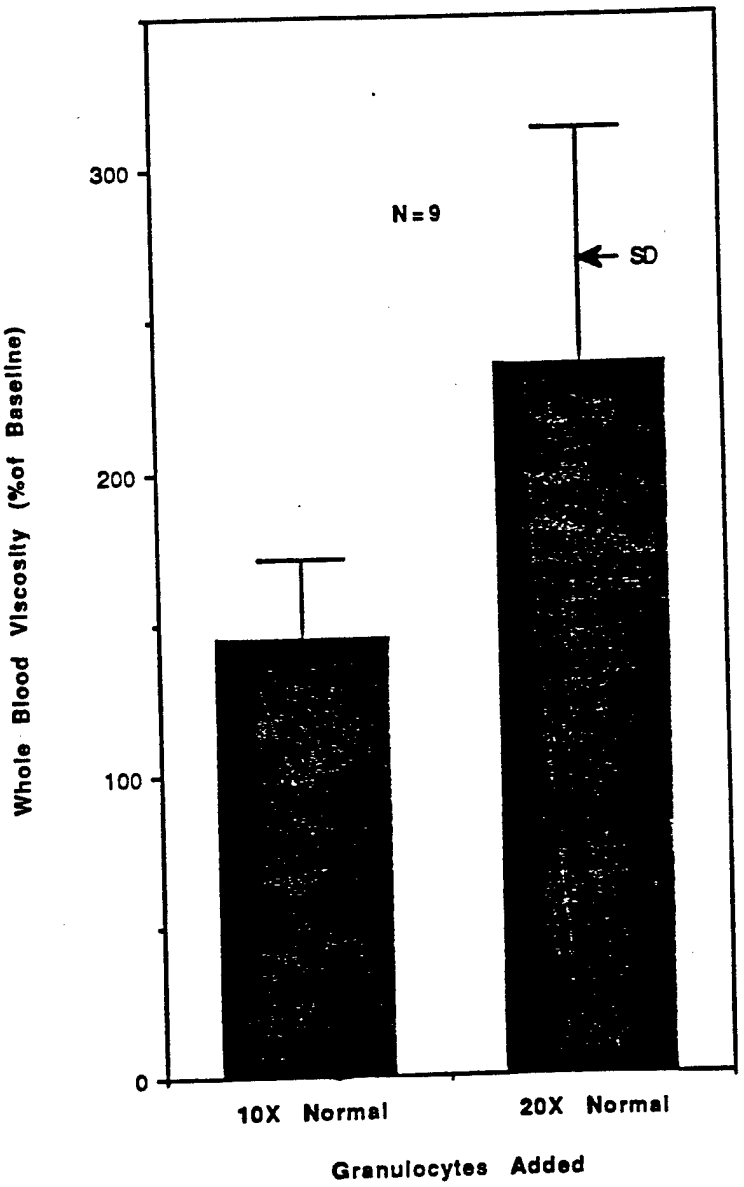

FIGURE 7: The effects of the addition to normal blood of mononuclear cells (lymphocytes and monocytes) obtained by leukopheresis and Ficoll-Hypaque separation.

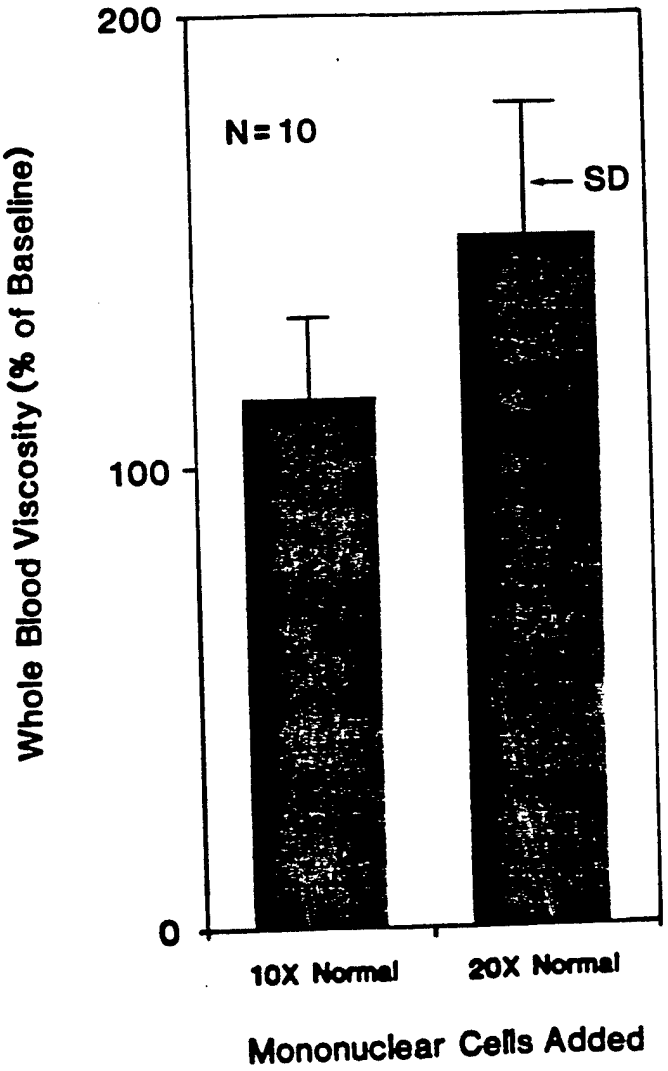

FIGURE 8: The effect of increasing WBC levels on WBV. Increased counts were achieved by the addition of either purified granulocytes or mononuclear cells obtained by leukopheresis then added to normal blood. PCV was kept constant at 43 ± 2 (range 40-45).

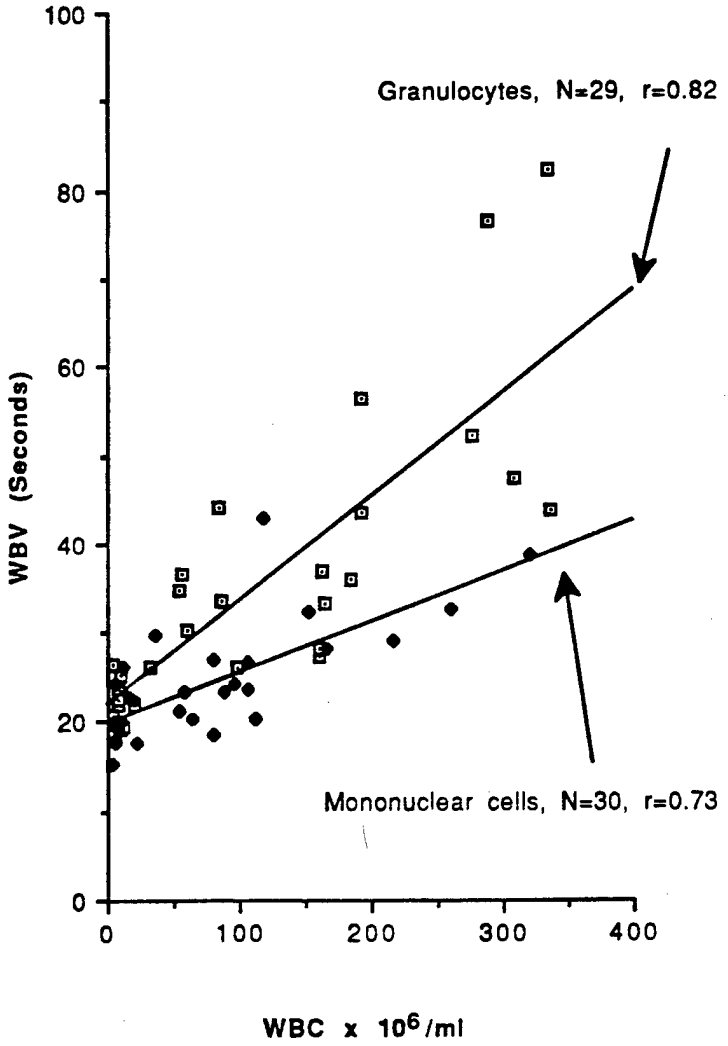

FIGURE 9: Relation of WBV to total PCV after the addition of granulocytes. PCV (RBC) was kept constant at 43 ± 2 (range 40-45).